

Bacterial Genetics

Introduction

Bacteria are really good at exchanging DNA. Like, REALLY good. They share it when they have sex, they share it when they get infected by a virus, and they can even share it after they die.

This lesson covers the mechanisms by which bacteria modify and share their DNA. It starts with plasmids and transposons (the DNA), then transitions into the specifics of conjugation, transformation, and transduction (sharing the DNA). After discussing the mechanisms, we introduce their clinical relevance.

Plasmids and Transposons

Plasmids are extrachromosomal, circular, double-stranded DNA molecules. The plasmids are capable of **replicating independently** of the bacterial chromosome, so one bacterium may have in its cytoplasm multiple copies of the plasmid DNA. And while they are usually extrachromosomal, plasmids are capable of being **integrated into bacterial chromosomal DNA**. Most importantly, plasmids can be transferred from cell to cell by conjugation (discussed next).

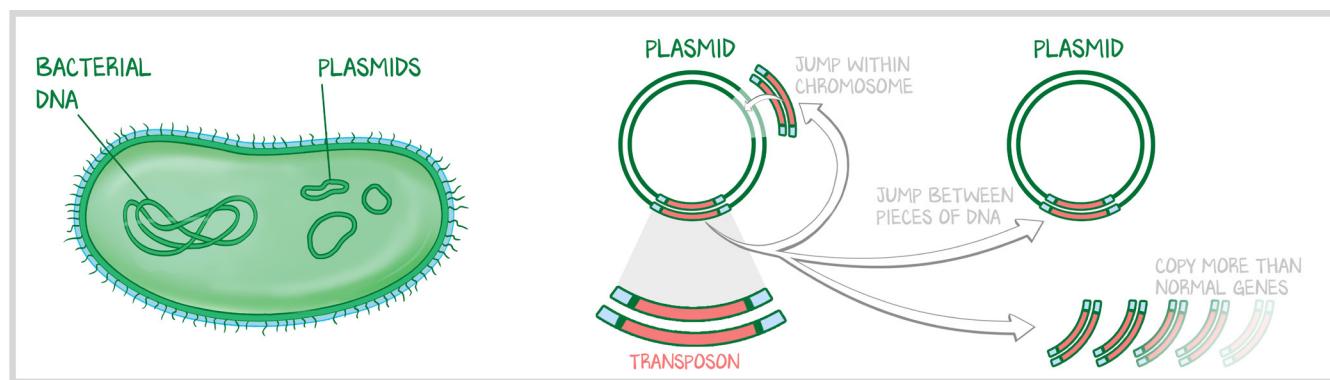


Figure 2.1: Plasmids and Transposons

Plasmids are circular extrachromosomal DNA that can be transcribed and synthesized, and can readily pass between bacteria. Transposons are stretches of DNA that readily move within the chromosome or between pieces of DNA (such as between a plasmid and the bacterial chromosomal DNA).

Transposons are pieces of DNA that can move readily from one site to another on a bacterial chromosome, or to or from a plasmid. The **transposon** is the string of DNA that moves. **Transposition** is the movement of pieces of DNA. Transposon DNA is copied, creating another transposon. That copy is excised and moved to another site. That other site might be within the chromosomal DNA or into another plasmid, or the copy might even exist within the plasmid it came from. A plasmid may contain multiple transposons and multiple copies of the same transposon. If each transposon carries its own mechanism for antibiotic resistance, and plasmids can be shared, transposons allow for the creation of plasmids with **multiple antibiotic resistances** that can be transferred easily between bacteria.

The most clinically relevant example of transposons and transposition is **transposon Tn1546**, which carries the **vanA gene**. Originally, the vanA gene was made by vancomycin-resistant enterococcus (VRE). Through transposition, the vanA gene, on transposon Tn1546, was transferred from VRE to *Staph. aureus*. Now we have vancomycin-resistant *Staph. aureus*.

Conjugation

Conjugation is the process by which DNA is transferred from **donor cells** that are F⁺ to recipient cells that are F⁻. This process is controlled by the **Fertility** gene, encoded on a Fertility plasmid.

The F⁺ cell forms a **mating pilus** under direction of the F gene. This mating pilus penetrates through the plasma membrane into the cytoplasm of an F⁻ cell, forming a **cytoplasmic bridge** between the donor F⁺ and the recipient F⁻ cells. DNA is double-stranded in bacteria. When the mating pilus forms, one of the two strands of plasmid DNA is dispatched across the mating pilus. That plasmid DNA is carrying the F gene. The recipient cell now has the plasmid with the F gene, and so is now F⁺. The recipient cell also receives any other genes on that plasmid. In this exchange of genetic material, the chromosomal DNA remains unchanged and untouched. The process ends with the breaking of the cytoplasmic bridge, the withdrawal of the sex pilus from the recipient cell. Both cells have a single strand of plasmid DNA. Both cells replicate the second strand using the single-stranded plasmid DNA as the template, restoring the double-stranded plasmid DNA in both cells. Conjugation is primarily responsible for **transfer of antibiotic-resistance genes**. Not only does conjugation give the recipient the ability to conjugate to another cell, but the conjugation and delivery of the plasmid brings with it useful genes. The recipient now carries the genes on that plasmid—both the antibiotic-resistance genes and the genes that allow for that bacterium to pass on that plasmid to other F⁻ cells.

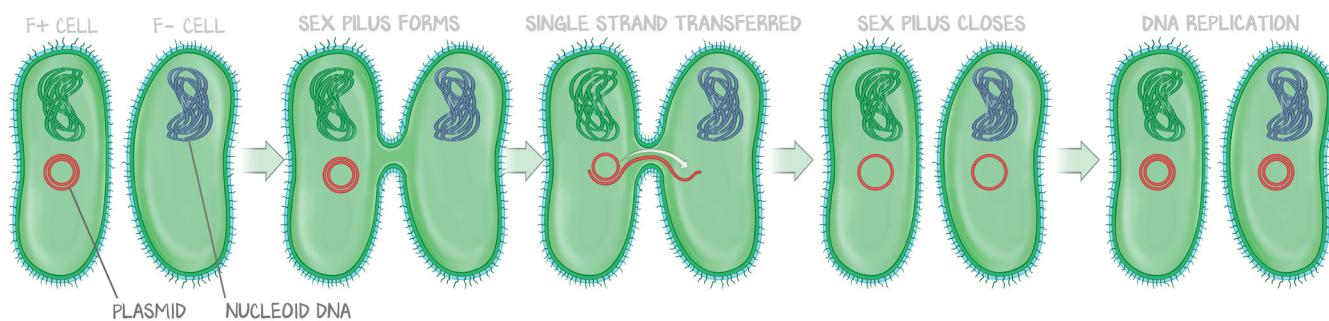


Figure 2.2: Conjugation

An F⁺ bacterium, one that possesses the plasmid gene for formation of the sex pilus, initiates conjugation with the extension of the sex pilus. Plasma membrane merge and a bridge of cytoplasm allows the F⁺ cell to send one strand of the DNA coding for the F⁺, located on a plasmid, across the sex pilus to the recipient cell. When the sex pilus closes, when the connection is broken, both have a single DNA strand of F⁺ plasmid. The double-stranded DNA is synthesized in both cells. The recipient is now F⁺ and has also acquired whatever genes were on the F⁺ plasmid. Chromosomal DNA is unaffected.

Transformation

When a bacterium dies, the plasma membrane ruptures and cytoplasm enters the extracellular matrix, and with it, the **naked bacterial chromosomal DNA**. Bacteria nearby possess specialized DNA-binding receptors on the extracellular face of their plasma membrane, which bind to a single strand of double-stranded DNA. Those receptors cleave one strand, which is degraded, and bring the remaining single strand into the cytoplasm. This new DNA pairs with one of the two homologous chromosomes inside the chromosomal DNA. Through **homologous recombination**, the single strand replaces its homolog. DNA-mismatch-repair enzymes recognize only that there are noncomplementary DNA base pairs at the location where the homolog entered the chromosome. Mismatch-repair enzymes don't know which strand was the original. In some instances, the repair enzymes excise the homolog and replace it with the original code. In some instances, the original DNA is excised and the homolog is used as the template to "fix" the DNA, resulting in the incorporation of the original dead bacterium's DNA into the new owner of that genetic code. Whichever version is excised gets degraded.

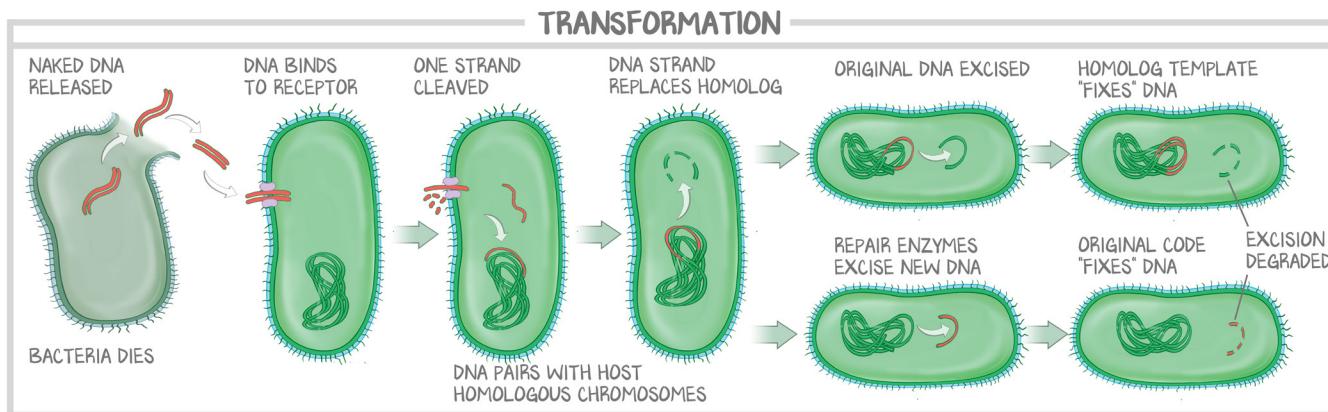


Figure 2.3: Transformation

When a bacterium dies, naked DNA is released into the surrounding environment. Using DNA receptors in the plasma membrane, still-living bacteria can pick up fragments of the dead bacterium's DNA. One strand is degraded; the other is brought into the cell, paired with one of its homologs in the chromosomal DNA, and incorporated into the chromosomal DNA. This results in a DNA mismatch, the new homolog not complementary and antiparallel to the original DNA. One of two things can happen: mismatch-repair enzymes excise the new DNA, or they excise the original DNA. If the original is excised, DNA polymerase uses the new DNA as a template, and the new genes are permanently added to the genome. If the new DNA is excised, DNA polymerase uses the old DNA as a template, and the original genes remain.

Test prep. In the lab, adding **deoxyribonucleases** (DNA nucleases degrade DNA) will degrade the naked DNA, preventing transformation. You may be given a failure to transform and be asked which enzyme is provided to prevent transformation, or you may be given the enzyme and asked to anticipate the outcome (failed recombination). A **stable** or **successful** transformation is one where the DNA fragment from one dead cell is integrated into the DNA of the recipient, and it lasts through cell division. An **unstable** or **failed** transformation is one where the DNA fragment from one dead cell is not found in the daughters of the original bacterium that would have taken up the naked DNA fragments.

Strep. pneumoniae, *H. influenzae*, and *Neisseria* (all species) are particularly well known to be good at transformation.

Transduction

Transduction is the transfer of a cell's DNA by means of a **virus**. The virus that does the transducing is called a **phage** or **bacteriophage**. For this section on transduction, “phage,” “virion,” and “virus” are used interchangeably, as you are not expected even to have seen viruses yet. In transduction in general, a virus infects a bacterium. A piece of bacterial DNA (there are two main mechanisms, discussed next) gets incorporated into a virus particle, which is carried by the phage to a different bacterium, whereby the phage injects the “viral DNA” into that new bacterium. But it’s not “viral DNA”—it is the originally infected cell’s DNA. The recipient cell just got donated a sample of new DNA it can keep or degrade.

There are two kinds of transduction—lytic infections blow apart the chromosome DNA and the cell while lysogenic infections are incorporated into chromosomal DNA, leaving the infected cell alive and well.

Lytic Phage, Generalized Transduction. Some viruses kill the cell they infect. They destroy the chromosomal DNA, they replicate until resources are depleted, and then they lyse the cell to release virions. These are **lytic viruses**. There is no integration of viral DNA into host DNA, and the host dies. Part of the virus life cycle is to use the infected cell to make more copies of itself. Another part is to use the infected cell machinery to build the protein coat (capsid) to stash the virus (genome) in for the next jump (together they are a virion). But because the chromosomal DNA is fragmented into little pieces and the viral DNA is multiple copies of little pieces (each strand another virus), sometimes the little

pieces of bacterial DNA get packaged into a capsid instead of the virus. The DNA is just the cargo of the phage particle. The phage particle is built correctly, it just gets packaged with the wrong DNA. So, when the phage goes to “infect” the next bacterium, it attaches, melds, and injects its contents into this next victim. But instead of a virus, it injects fragments of bacterial DNA that does not code for any of the virus stuff. This bacterium is safe from infection (at least with this phage injection), because there is no virus. And instead of a virus, this bacterium now has access to new DNA, which can be incorporated into its chromosomal DNA or simply degraded. Because the originally infected bacterium had its chromosomal DNA fragmented, any part of the fragmented chromosomal DNA could be packaged; there is equal chance of any DNA getting transferred. This is called **generalized transduction** because it is NOT specific DNA that is transferred.

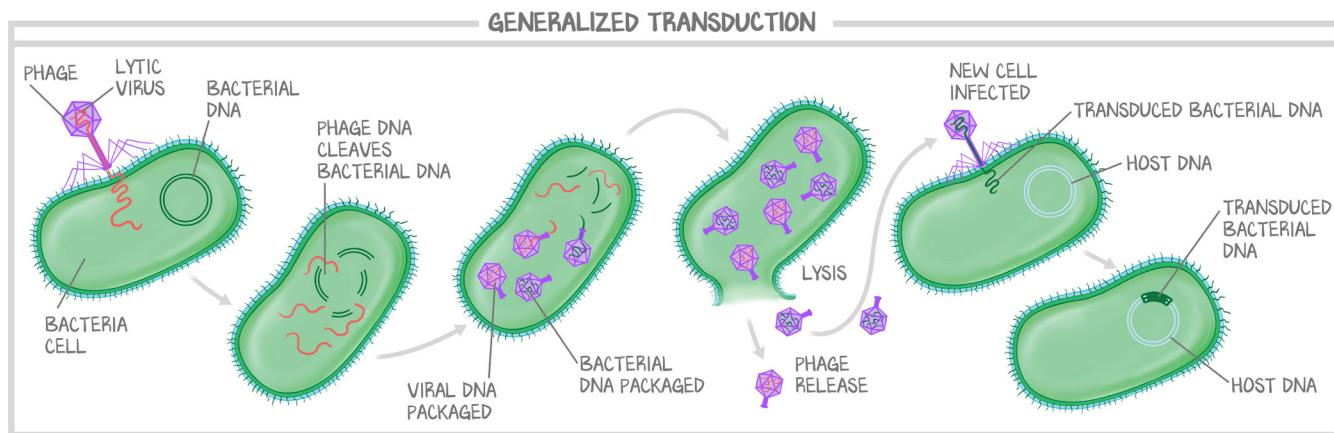
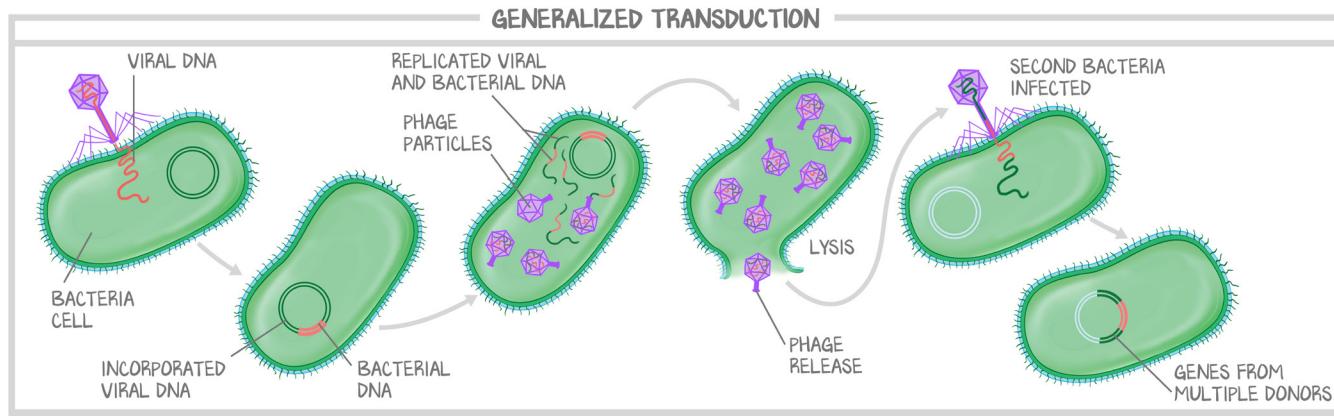


Figure 2.4: Generalized Transduction

Generalized transduction is the inevitable outcome of an infection with a virus that undergoes a lytic infection cycle. The host cell is destroyed, and its DNA degraded. The host cell DNA fragments may be mistaken as viral genome, and are packaged in virions to “infect” the next cell. Because the DNA is fragmented as it is degraded, any bacterial DNA can be taken up into a phage because the entire chromosome is degraded (opposed to specialized transduction, discussed next).

Lysogenic Cycle, Specialized Transduction. Some viruses DO NOT kill the cell they infect. Instead, they **integrate** their viral DNA into chromosomal DNA. This is much less disruptive to the infected cell. The viral DNA becomes host DNA. The cell does not die. The viral DNA still hijacks host machinery (see the Virus series for more details) to replicate multiple copies of itself, make the proteins to infect other cells, package the phages, and release them outside of the cell. The virus still hijacks the host’s resources, but this does not result in cell death. Because the DNA is not all chopped up, only the viral DNA will be incorporated into phages. The only “mistake” that can be made is that any copies of the virus that get made could include more than just the viral genome. The genes flanking the viral DNA can be copied and transferred into the phage along with the viral DNA. When the virus infects the next bacterium, the virus gets incorporated into the host DNA. “The virus” this time was actually “virus plus extra bacterial DNA.” That extra DNA could be coding domains for virulence proteins, such as an exotoxin. Five bacterial toxins are coded in a lysogenic phage: Group A strep, Botulinum toxin, Cholera toxin, Diphtheria toxin, and Shiga toxin (ABCDs).

**Figure 2.5: Specialized Transduction**

Specialized transduction requires lysogenic infections where the host cell and DNA are maintained. The virus incorporates itself into the host DNA. When new viral genome is replicated, host DNA flanking the virus may be replicated as well, resulting in transmission of that flanking region into the phage.